Using modifications to the *in vitro* motility assay under unloaded and loaded conditions, we examined the biochemical properties of two RLC mutations that have been implicated in causing familial hypertrophic cardiomyopathy, N47K and R58Q. Myosin was purified from porcine ventricles carrying beta-MHC, and the native RLC was replaced with recombinant human wild type (WT) or mutant RLC. Our data show that under unloaded conditions, there are no differences between the mutant myosins and the WT. On the other hand, consistent with skinned fiber studies, we saw significant changes under loaded conditions, with both mutants showing reductions in isometric force, power output, and the load at which peak power occurs. We also see that both ATP and ADP affinity are load-dependent in the WT. Interestingly, our data suggests that cardiac myosin undergoes a load-dependent isomerization in the ADP bound state, similar to other "load sensing" myosins. Furthermore, we show that whereas WT shows a reduction in affinity for exogenously added ADP under loaded conditions, the mutants are relatively insensitive to load. Taken together these data demonstrate that mutations of the RLC change the load dependent kinetics of cardiac myosin, suggesting a role for the RLC in tuning load-dependent myosin mechanochemistry.

1124-Plat
Gravitational Force Spectroscopy Reveals Separation of Myosin Heads at the S1/S2 Hinge
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In order for striated muscle myosin to interact productively with thin filaments, it must both span the gap between thin and thick filaments and possess sufficient degrees of freedom to align its actin binding site with protomers of various helical orientations. This flexibility requires extensions and rotations of the coiled coil S2 domain of myosin including the S1/S2 hinge. To examine the ability of the coiled coil to separate, a novel gravitational force spectrometer was created that can apply femtonewton to piconewton forces with high accuracy to regions of a single myosin molecule defined by site-specific antibodies. Force-distance curves indicate that when the piconewton forces are applied perpendicular to the long axis of the S2 coiled coil at the S1/S2 hinge, the strands separate readily in a force dependent manner. However when similar levels of force are applied parallel to the long axis of the S2 coiled coil, there is much less extension. Computational force spectroscopy simulations on the atomic model of human S2 provided confirming results and with atomic resolution detail. As a control, simulations on scallop myosin S2 indicated that its coiled coil separates with less force than human S2 which is consistent with previously published reports. Furthermore, several familial hypertrophic cardiomyopathy causing mutations in the S1/S2 hinge were introduced into the human S2 simulations and resulted in further destabilization of its nanomechanical properties. These results indicate that the myosin S2 has intrinsic structural functions that may be independent of its interactions with other proteins. It is possible that interactions with myosin binding proteins may modulate these properties. (Supported by NSF 084273 ARRA)

1125-Plat
Three-Dimensional Structure of the Relaxed State of Calcium-Regulated Myosin Filaments
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Myosin filaments of muscle are regulated either by phosphorylation of their regulatory light chains or Ca^{2+}-binding to the essential light chains, contributing to on-off switching or modulation of contraction. Phosphorylation-regulated filaments in the relaxed state are characterized by an asymmetric interaction between the "blocked" and "free" heads of each myosin, inhibiting actin-binding or ATPase activity (Wendt et al., 2001; Woodhead et al., 2005). We have tested whether a similar interaction occurs in Ca^{2+}-regulated filaments. Filaments were purified from scallop striated adductor muscle and imaged by cryo-electron microscopy. 3D reconstruction was carried out by single particle methods. Reconstructions showed a 7-fold symmetric, helical array of myosin head-pair motifs lying above the filament surface. Fitting of the motif with a myosin head atomic model revealed that the heads interact in a similar way to phosphorylation-regulated filaments. However, the 2-headed motif was more tilted and higher above the filament surface in the Ca^{2+}-regulated filaments. Subfragment 2 of the myosin tail emerged from the motif near the blocked head and connected the motif to the filament backbone, which comprised a 7-fold array of comma-shaped subfilaments. This structure reveals new detail compared with a previous cryo-EM study (Vibert, 1992) and demonstrates that the interpretation of head organization in a negative stain reconstruction of scallop filaments (AL-Khayat et al., 2009) is incorrect. We conclude that the relaxed state of Ca^{2+}-regulated filaments is achieved in a similar way to phosphorylation-regulated filaments, confirming that head-head interaction is a widely used motif (Woodhead et al., 2005). In the scallop filament, the pairs of myosin heads are much closer together azimuthally and the subfilaments have a different structure compared with phosphorylation-regulated filaments, implying that general models for thick filament structure (Squire, 1973) need modification. Supported by NIH grant AR34711.

1126-Plat
Electron Tomography of Cryofixed, Isometrically Contracting Insect Flight Muscle Reveals Novel Actin-Myosin Interactions
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We have applied multivariate data analysis to 38.7 nm repeat segments obtained from electron tomograms of isometrically contracting insect flight muscle fibers, mechanically monitored, rapidly frozen, freeze-substituted and thin sectioned. Improved resolution reveals for the first time the helix of F-actin subunits in the thin filament with sufficient clarity that an atomic model can be built into the density independent of the myosin cross-bridges, thereby providing an objective method for identifying weak and strong actin-myosin attachments. The tomogram shows strong binding myosin attachments on only four F-actin subunits midway between successive troponin complexes; these actin subunits comprise the "target zone" of active contraction. Improved quantitation facilitates a more detailed description of weak and strong myosin attachments all along the thin filament including for the first time myosin heads contacting the thin filament on troponin. Most strong binding actin attachments consist of single myosin heads but 28% of bound heads are 2-headed myosin attachments. Strong binding attachments show an axial lever arm range of 77° sweeping out a distance of 12.9 nm. The azimuthal range for the lever arm of strong binding attachments is 127° with a distribution nearly completely to one side of the initial crystallographic structures used for the fitting. There is no apparent coupling between axial angle, representing progress through the power stroke of myosin, and the azimuthal lever arm angle. Two types of weak actin attachments are observed. One type, which is found exclusively on target zone actin subunits, appears to represent prepowerstroke intermediates. The other, which appears to have a different function, is positioned on the M-ward side of the target zone, i.e. the direction toward which filaments slide during sarcomere shortening. Its motor domain contacts tropomyosin rather than actin. Supported by NIH.

1127-Plat
Microscopic Measurement of Periodic Cross-Bridge Formation in Skeletal Myofibril
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Electron microscopy and X-ray diffraction studies have been usually used for the examination of the interactions between actin and myosin, i.e., cross-bridge formation, in muscle fibers. These studies suggested the existence of "target zones [regions]" in actin (thin) filament, which are composed of three to four actin monomers that myosin heads in myosin thick filament can form cross-bridges. Direct evidence for the target zones, however, has been missing in myofibrils. Here we studied the interaction between a single actin filament and the thick filaments of rabbit skeletal myofibril under optical microscope. Single bead-tailed actin filament was manipulated by optical tweezers to make rigor cross-bridges on the outer surface of myofibril, and rupturing events were directly detected. We found the periodic cross-bridge formation, and frequently observed gaps, which are probably due to the incommensurate helical pitches between the thin and the thick filaments.